THE PRODUCTION OF ACID BY THE BACILLUS COLI GROUP *

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The year 1885 marks the beginning of the study of the bacteria of the intestinal tract in relation to their action on the various carbohydrates. In that year, Buchner, working with his "Darmbacillus G," which, in all probability, was a member of the bacillus coli group, found that growth in a medium, consisting of meat infusion, peptone, and sugar, was accompanied by the formation of acid and gas. The acid was demonstrated by the addition of litmus to the medium. The production of acid and gas, according to Buchner, was due to the breaking down, or decomposition, of the sugar brought about by the action of the bacteria. The evolved gas was found to be carbon dioxid, and the acids were defined as members of the fatty acid series. The work of Buchner may be considered as the starting point of all the work done on the relation of bacteria to the decomposition of carbohydrates with the production of acid and gas.

In connection with the production of acid in carbohydrate solutions by bacteria, progress has been particularly active along two distinct lines: (1) The differentiation of the colon group from the typhoid group and other alkali producers, and (2) the classification of the bacillus coli group, according to the ability of its members to ferment the various carbohydrates.

Petruschky's litmus whey medium, Beijerinck's calcium carbonate medium, Wurty's litmus lactose agar, Kaufmann's jequirity solution, and Hanna's proteid medium were important steps in the investigations along the former line. Among the workers of this period, who have contributed to our knowledge of carbohydrate fermentation, are Beginsky (1888), Lembke (1896), Von Sommoruga (1892), Capaldi and Proskauer (1897), Ziellecyky (1902) and Segin (1903). These results were augmented by Theobald Smith by use of the fermentation tube.

Beginning with the work of Durham in 1900, nearly all the investigations along the line of fermentation of carbohydrates by the bacillus coli group have been done with the idea of classifying the members of the group by means of their fermentative reactions on the various carbohydrates. The work of Dur-

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ham has been extended and augmented by the notable researches of Houston (1902), MacConkey (1905), Winslow and Walker (1907), Graham Smith (1909), and Jackson (1911).

In spite of the importance of this fermentative reaction, however, there are many of the finer points of technic which have never been fully and conclusively worked out. Acid production, like all other biochemical reactions, is a function of the reacting organism, the substance decomposed, the end products formed, the time, the temperature, and all conditions which may check or favor the vital process. In the present investigation, I have attempted to isolate these various factors and study them, one by one, with the idea of gaining a clear idea of the quantitative significance of each. No startlingly novel conclusions could be expected, but it is hoped that the data obtained may furnish a surer basis for comparative studies of fermentative power in carbohydrate media than has been available in the past.

ORGANISMS USED IN THE INVESTIGATION

The organisms used in this work were all members of the bacillus coli group. Altho the identification of this group has been a subject of dispute for over a quarter of a century, it is now generally conceded that the following characteristics may be considered criteria of membership: (1) Short bacillus with rounded ends; (2) gram-negative; (3) non-liquefaction of gelatin in 16 days; (4) fermentation of dextrose and lactose with the production of acid and gas; (5) non-spore-forming; (6) facultative anaerobe; (7) gas in lactose-peptone-bile; (8) grayish white growth on agar at 20 C. and 37 C.

Other characteristic properties of this group, such as the reduction of nitrates to nitrites, production of indol, motility, and style of growth on various media, are valuable, if at all, merely for establishing minor subdivisions.

The organisms used in this investigation all conformed to the general characteristics of the bacillus coli group, as noted. In the early part of the work, no particular attention was paid to the subdivisions of the group, and, as a result, organisms were used which were identified only by the general group characteristics. As the work developed and the number of carbohydrates in use increased, the subdivisions of the group began to manifest themselves. A clean-cut differentiation into various species was obtainable by the production

or non-production of acid in the carbohydrate solutions. As the writer had no dulcite at hand, a subdivision of the group into species, as recommended by Jackson, was impossible, and, as a result, all the strains used throughout the work have been designated as the bacillus coli, altho it is certain that a number of species were present. As far as possible, those organisms which gave similar fermentative reactions have been grouped in the same table.

The cultures were obtained from two sources; either from oysters grown in contaminated water, or directly from feces. The oysters, from which the organisms were isolated, were taken from 242 different stations in Narragansett Bay during an investigation to determine the amount of pollution of the oyster-beds of the State of Rhode Island. The area from which the oysters were taken included localities, varying from those extremely polluted near sewer outlets to the less polluted areas, which were sometimes coli-positive and other times coli-negative.

One series of the cultures, isolated from feces, was obtained from normal stools of people in and about the laboratory at Brown University, while the other series was obtained from the stools of Italian immigrants, quarantined aboard the *S. S. Roma,* who were undergoing examinations for the cholera vibrio. These two series were kept entirely distinct and will be so designated throughout the work.

METHODS

Preparation of Culture Media.—The media were prepared after the methods proposed in the Report of the Committee on Standard Methods of Water Analysis to the Laboratory Section of the American Public Health Association, January 5, 1905, Liebig's meat extract was used, 3 grams to the liter. Ease of preparation rendered it more suitable for the work of this kind than a medium made up with meat. Great care was taken in the preparation of the media to make all the lots as uniform as possible. For this reason, all the constituents were taken from the same lot of materials during the entire course of the experiments.

The carbohydrate solutions were made by the addition of 1 percent of the various carbohydrates to neutral nutrient broth. Twenty-five cubic centimeters of the broth were placed in regular laboratory test tubes of large size, and sterilized on three successive days in streaming steam at 100 C.

During the course of the experiments, the production of acid was determined in the following carbohydrates:

I. I. Monosaccharids:

A. Hexoses.
   1. Dextrose (Merck).
   2. Galactose (Merck).
   3. Levulose (Kahlbaum).

B. Pentoses.
   1. Arabinose (Kahlbaum).
   2. Xylose (Kahlbaum).

II. Disaccharids:
    1. Lactose (Merck).
    2. Maltose (Merck).
    3. Saccharose (Merck).
III. Trisaccharid:
    1. Raffinose (Kahlbaum).
IV. Hexatomic alcohols:
    1. Iso-dulcite (Kahlbaum).
    2. Mannite (Merck).

All cultures used in the determination of the production of acid in the carbohydrate solutions were grown at 37 C. for 24 hours, unless otherwise stated. The titrations were made with N/20 sodium hydroxid at the twenty-fourth hour, and results tabulated in direct percentages of normal sodium hydroxid.

Method of determining amount of acid produced.—From a twenty-four-hour agar slant culture of the organism, which had been identified as a member of the bacillus coli group, inoculations were made into peptone solutions, which were incubated for 24 hours at 37 C. At the end of 24 hours, the tubes of carbohydrate solution were inoculated by the addition of 0.5 c.c. of the twenty-four-hour peptone culture. By the addition of a definite amount of the twenty-four-hour culture, more consistent results were obtained than by the direct inoculation from the agar slant culture.

The inoculated carbohydrate solutions were incubated for 24 hours and were then ready for titration. The cultures were titrated as soon as possible after their removal from the incubator.[1]

Five cubic centimeters of the culture and 45 c.c. of distilled water were placed in a caserole and boiled briskly for one minute. One cubic centimeter of phenolphthalien, which consisted of 5 gm. of the commercial salt dissolved in one liter of 50 percent alcohol, was added as an indicator. Titrations were made into the hot solution until a faint, but permanent, pink color was obtained with N/20 sodium hydroxid solution.

THE RELATION OF TEMPERATURE TO THE AMOUNT OF ACID PRODUCED

Twenty-five cubic centimeters of the various sterilized carbohydrate solutions were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the identified organisms. The cultures and their controls were kept for 24 hours at the following temperatures: Ice-box temperature, 3 C.; room temperature, 16 C.; incubator temperature, 28 C.; incubator temperature, 37 C.; incubator temperature, 45 C.

At the end of the twenty-fourth hour the cultures and their controls were titrated as soon as accuracy would allow. All titrations were made with N/20 sodium hydroxid, and all results are expressed in direct percentages of normal sodium hydroxid.

The results obtained in these experiments show that cultures of the bacillus coli group, grown at a temperature of 37 C., tend to produce more acid in carbohydrate solutions, in a given time, than cultures grown at other temperatures. Cultures grown at 3 C. show almost no acid production in 24 hours. As we approach 37 C., either from below

1. The method of titration followed was the one outlined in Standard Methods of Water Analysis, 1905, p. 106.

TABLE 1

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED AT DIFFERENT TEMPERATURES BY BACILLUS COLI, ISOLATED FROM FECES

Temperature	Percentage of Acid with Dextrose	Percentage of Acid with Lactose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Maltose	Percentage of Acid with Xylose	Percentage of Acid with Arabinose	Percentage of Acid with Mannite	Percentage of Acid with Isodulcite	Percentage of Acid with Control
3 C.	0	0	0	0	0	0	0	0	0	0
16 C.	0.6	0.3	0.7	0.3	0.3	0.3	0.3	0.3	0.2	0
28 C.	2.0	1.7	2.1	1.3	1.6	1.8	1.7	1.7	1.7	0
37 C.	2.3	1.9	2.3	1.9	1.9	2.0	2.0	2.0	2.2	0
45 C.	1.3	1.0	1.5	0.9	0.3	1.3	1.2	1.0	0.2	0

The results are the average of the titrations of two cultures.

TABLE 2

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED AT DIFFERENT TEMPERATURES BY THE BACILLUS COLI, ISOLATED FROM OYSTERS

Temperature	Percentage of Acid with Dextrose	Percentage of Acid with Lactose	Percentage of Acid with Saccharose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Maltose	Percentage of Acid with Raffinose	Percentage of Acid with Xylose	Percentage of Acid with Arabinose	Percentage of Acid with Mannite	Percentage of Acid with Isodulcite	Percentage of Acid with Control
3 C.	0	0	0	0	0	0	0	0	0	0	0	0
16 C.	0.4	0.1	0.1	0.6	0.2	0.3	0.2	0.3	0.3	0.3	0.2	0
28 C.	2.0	1.7	1.4	2.1	1.2	1.6	0.9	1.6	1.6	1.4	0.8	0
37 C.	2.2	2.0	1.4	2.3	1.8	1.9	1.5	1.9	2.0	1.9	2.0	0
45 C.	1.2	0.7	0.3	1.3	0.8	0.2	0.2	1.2	1.0	0.9	0.2	0

The results are the average of the titrations of two cultures.

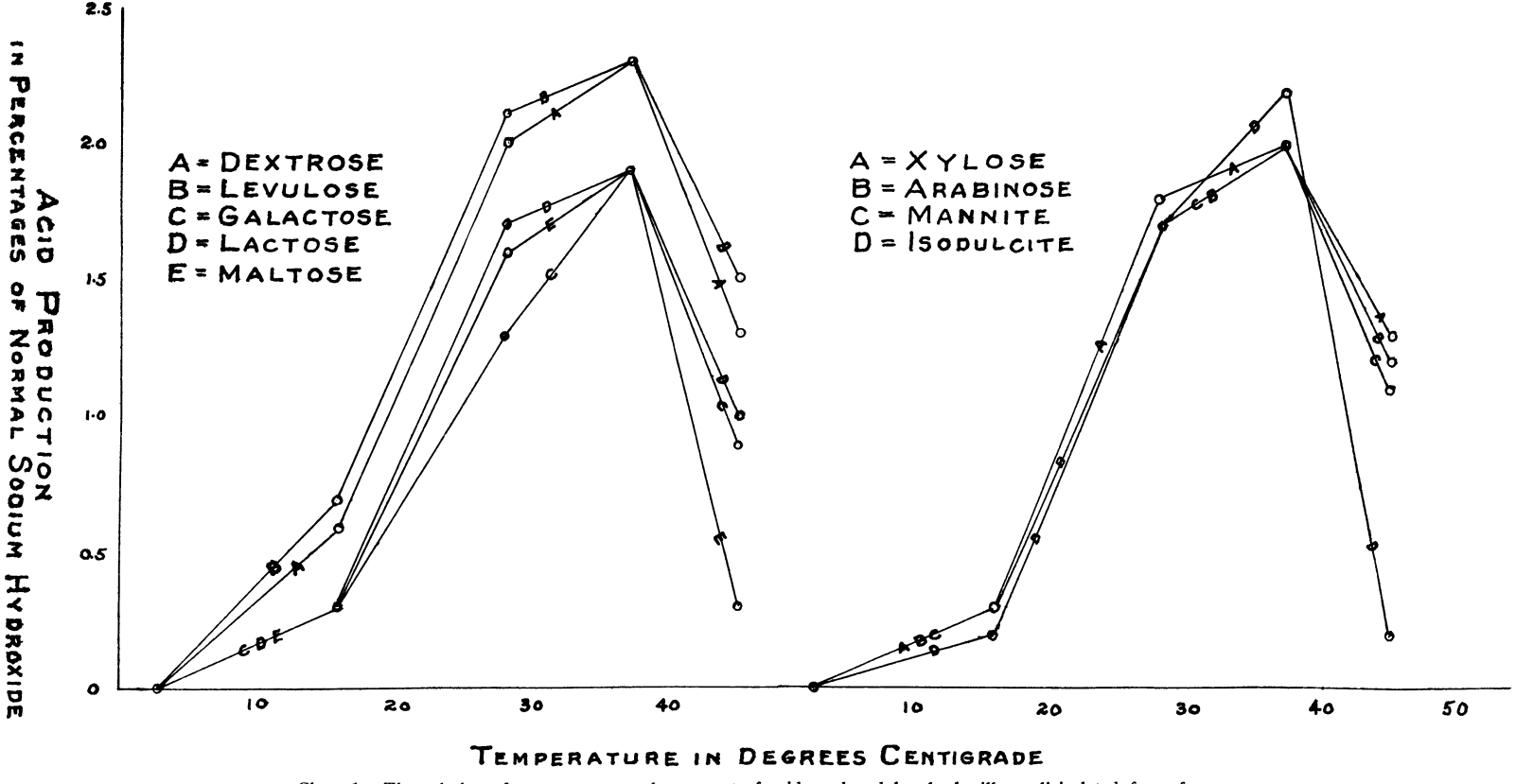

Chart 1.—The relation of temperature to the amount of acid produced by the bacillus coli, isolated from feces.

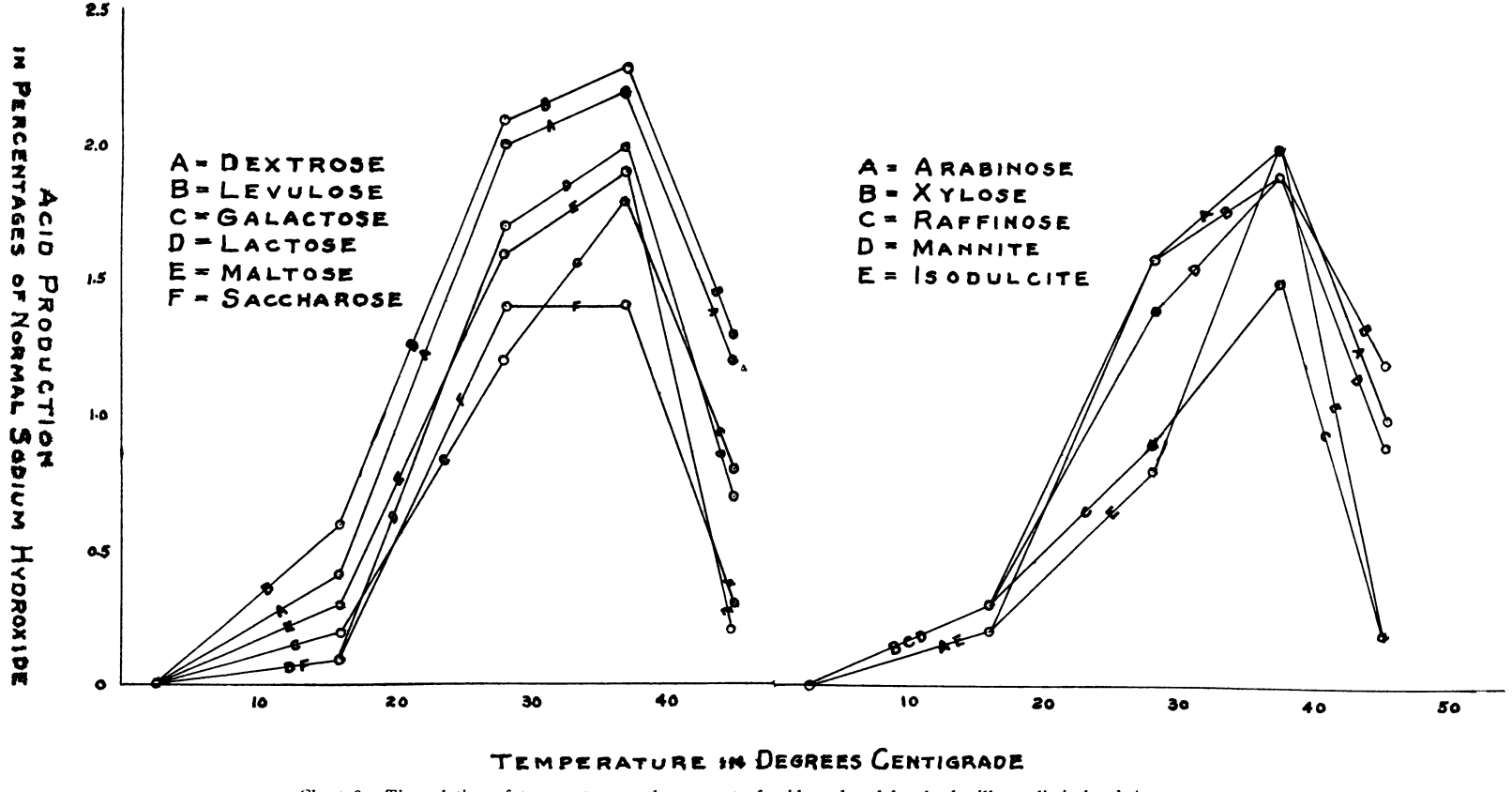

Chart 2.—The relation of temperature to the amount of acid produced by the bacillus coli, isolated from oysters.

or above, the amount of acid increases until the maximum amount of acid is produced at 37 C. We should expect this, since 37 C. is the optimum temperature for the growth of the bacillus coli group. If the temperature is too low or too high, we get less growth, and since acid production is dependent on the growth of the organism, we must surely find the optimum temperature for the production of acid identical with the optimum temperature for growth. This was found to be true in all the carbohydrate solutions used.

THE RELATION OF TIME TO THE AMOUNT OF ACID PRODUCED IN VARIOUS CARBOHYDRATE MEDIA

The general method of these experiments consisted in the inoculation of a large number of tubes of the 1 percent carbohydrate media with various members of the coli group. At definite intervals, a certain number of the tubes were removed from the incubator and titrated with N/20 sodium hydroxid. These experiments include 10 carbohydrates using various members of the bacillus coli group. Fifteen tubes of the 10 carbohydrates noted below, each containing 25 c.c. of the medium, were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the bacillus coli. The tubes were incubated at 37 C., and were removed from the incubator and titrated at intervals of three hours.

From the results obtained in these experiments it is seen that, by the end of the twenty-fourth hour, the members of the bacillus coli group produce their maximum amount of acid when grown at 37 C., after the inoculation of a sugar medium with 0.5 c.c. of a twenty-four-hour peptone culture. In the case of some carbohydrates, such as dextrose or lactose, the maximum production of acid occurs in the eighteenth hour. The organisms used in this experiment produced their maximum amount of acid either before or at the twenty-fourth hour. Cultures, which were grown for 48 and 72 hours at 37 C., showed no increase over the amount of acid produced at the end of the twenty-fourth hour. The maximum amount of acid was produced by the organisms by the end of the twenty-fourth hour.

THE RELATION OF THE AMOUNT OF ACID PRODUCED TO THE AMOUNT OF MEDIUM INOCULATED

Erlenmeyer flasks, containing 25, 50, 100, 200, 300, 400, and 500 c.c. of dextrose and lactose broths, were inoculated with 0.5 c.c. of a twenty-four-hour culture of the coli bacillus. The flasks were incubated for 24 hours at 37 C., and then titrated with N/20 sodium hydroxid.

TABLE 3

ACID PRODUCTION, IN PERCENT NORMAL, AT INTERVALS OF THREE HOURS, BY THE BACILLUS COLI, ISOLATED FROM FECES

Hours	Percentage of Acid with Dextrose	Percentage of Acid with Lactose	Percentage of Acid with Saccharose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Maltose	Percentage of Acid with Raffinose	Percentage of Acid with Arabinose	Percentage of Acid with Isodulcite	Percentage of Acid with Mannite
3	1.0	0.6	0.35	0.95	0.35	0.7	0.35	0.5	0.35	0.35
6	1.9	1.4	0.3	1.8	0.7	0.8	0.7	1.7	1.1	1.3
9	2.2	1.9	0.4	1.9	0.85	1.05	1.15	1.9	1.6	1.3
12	2.35	1.9	1.1	2.1	1.3	1.7	1.3	2.0	1.9	1.3
15	2.4	1.9	1.85	2.2	1.9	1.8	1.35	2.1	1.9	2.0
18	2.4	1.9	2.0	2.3	1.9	2.0	1.7	2.2	2.2	2.0
21	2.4	1.9	2.0	2.3	2.1	2.0	2.0	2.2	2.2	2.0
24	2.4	1.9	2.0	2.2	2.1	2.0	2.0	2.2	2.1	2.0
42	2.4	2.0	2.1	2.3	2.1	2.0	1.9	2.2	2.1	2.0

All titrations are the average of titrations of two cultures.
The controls titrated at the end of 24 hours gave a neutral reaction.
The temperature was 37 C.

TABLE 4

ACID PRODUCTION, IN PERCENT NORMAL, AT INTERVALS OF THREE HOURS, BY THE BACILLUS COLI, ISOLATED FROM OYSTERS

Hours	Percentage of Acid with Dextrose	Percentage of Acid with Lactose	Percentage of Acid with Saccharose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Maltose	Percentage of Acid with Raffinose	Percentage of Acid with Arabinose	Percentage of Acid with Isodulcite	Percentage of Acid with Mannite
3	0.45	0.35	0.15	0.4	0.35	0.4	0.35	0.35	0.3	0.2
6	1.5	0.8	0.9	1.4	1.0	1.0	0.4	1.5	1.0	1.0
9	1.6	0.8	1.7	1.8	1.35	1.05	0.4	1.4	1.5	1.1
12	1.9	0.8	1.7	1.9	1.7	1.3	0.4	1.4	1.8	1.2
15	1.9	1.45	1.8	1.9	1.7	1.3	0.9	1.4	2.0	1.3
18	1.9	1.5	1.6	2.1	1.8	1.3	1.4	1.5	2.2	1.3
21	1.9	1.5	1.6	2.1	2.1	1.4	1.4	1.6	2.2	1.3
24	2.0	1.9	1.6	2.0	2.1	1.4	1.5	1.6	2.1	1.3
42	2.0	1.9	1.6	2.0	2.1	1.4	1.6	1.7	2.1	1.3

All titrations are the average of the titrations of two cultures.
The controls were titrated at the end of 24 hours and the reaction was neutral.
The temperature was 37 C.

The results obtained show that no matter how much carbohydrate media is inoculated, up to 500 c.c., the same percentage of acidity is produced by the bacillus coli when grown at 37 C. for 24 hours after inoculation with 0.5 c.c. of a twenty-four-hour peptone culture.

THE RELATION OF THE CONCENTRATION OF THE CARBOHYDRATE MEDIUM TO THE AMOUNT OF ACID PRODUCED

Tubes of neutral nutrient broth, to which were added various percentages of dextrose and lactose, were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the bacillus coli. The tubes were incubated for 24 hours at 37 C., and at the twenty-fourth hour the tubes were titrated with N/20 sodium hydroxid.

TABLE 5

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED BY THE BACILLUS COLI ISOLATED FROM FECES, IN NEUTRAL BROTH CONTAINING VARYING PERCENTAGES OF DEXTROSE AND LACTOSE

Percentage of Dextrose	Amount of Acid Produced Percent	Percentage of Lactose	Amount of Acid Produced Percent
0.125	0.4	0.125	0.35
0.25	0.5	0.25	0.5
0.5	2.1	0.5	1.7
0.1	2.2	1	1.8
1.5	2.2	1.5	1.8
2	2.3	2	1.8
2.5	2.2	2.5	1.75
3	2.4	3	1.8
3.5	2.3	3.5	1.8
4	2.2	4	1.8
4.5	2.3	4.5	1.8
5	2.4	5	1.8
7.5	2.35	7.5	1.7
10	2.3	10	1.8
15	2.3		
20	2.3		
25	2.3		
30	1.8		
35	0.9		
40	0.8		
45	0.6		
50	0.3		

The results are the average of the titrations of two cultures.
Temperature at 37 C.

From these experiments it appears that the concentration of the carbohydrates (dextrose and lactose) has little effect on the amount of acid produced, within certain limits, by the bacillus coli. Between 1 percent and 25 percent concentration, the amount of acid produced is nearly constant. Below 1 percent and above 25 percent there is less acid produced. A 1 percent solution gives the maximum amount of acid from the least amount of the carbohydrate.

THE RELATION OF THE INITIAL REACTION OF THE CARBOHYDRATE MEDIUM TO THE AMOUNT OF ACID PRODUCED

The purpose of these experiments was to determine whether or not the initial reaction of the carbohydrate medium had any influence upon the production of acid by the organisms.

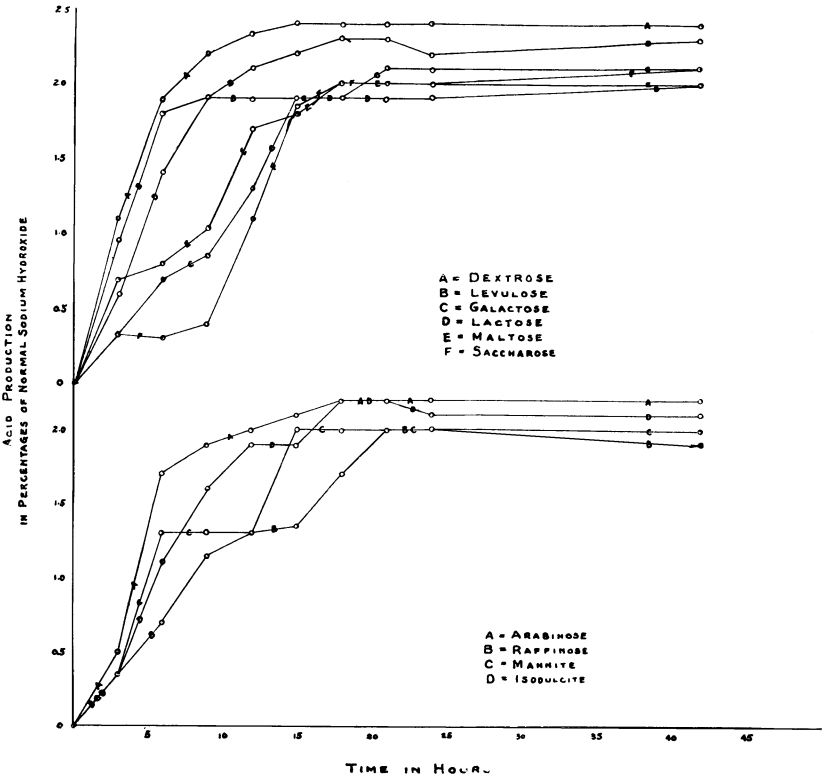

Chart 3.—The relation of time to the amount of acid produced by the bacillus coli, isolated from feces.

Tubes of dextrose broth, to which varying amounts of sterile acid and alkali were added after sterilization, were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the bacillus coli. These tubes were incubated for 24 hours at 37 C. At the end of 24 hours the cultures were titrated with N/20 sodium hydroxid.

The amount of acid produced by the bacillus coli group, in various carbohydrate media, depends, in great part, upon the initial reaction of the medium. The maximum acid production of an organism is the

TABLE 6

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED IN DEXTROSE BROTH, WITH VARYING INITIAL REACTIONS, BY THE BACILLUS COLI, ISOLATED FROM FECES AND FROM OYSTERS

Initial Reaction of Dextrose Broth	Amount of Acid Produced by Colon Bacilli from Feces					Amount of Acid Produced by Colon Bacilli from Oysters				
	Culture 1	Culture 2	Culture 3	Average	Control	Culture 1	Culture 2	Culture 3	Average	Control
+4.4	4.4	4.5	4.4	4.42	4.3	4.3	4.3	4.3	4.3	+4.4
+2.65	2.6	2.7	2.7	2.66	2.65	2.5	2.5	2.5	2.5	+2.6
+2.1	2.3	2.4	2.3	2.33	2.0	2.3	2.3	2.3	2.3	+2.1
+1.0	2.3	2.3	2.3	2.3	1.0	2.5	2.5	2.5	2.3	+1.0
+0.15	2.2	2.3	2.3	2.26	0.1	2.3	2.3	2.3	2.3	+0.15
—0.5	2.3	2.3	2.4	2.33	0.5	2.4	2.4	2.4	2.4	—0.5
—0.6	2.3	2.3	2.3	2.3	0.6	2.4	2.4	2.4	2.3	—0.6

Temperature was 37 C.

amount of acid necessary to prevent further growth. The farther away the initial reaction is from the maximum acid production, the more acid an organism can produce until it reaches the maximum. The limiting acidity of the bacillus coli group is about 2.4 percent normal sodium hydroxid. A medium with a slightly acid reaction offers a shorter path to travel to the maximum acidity than a medium

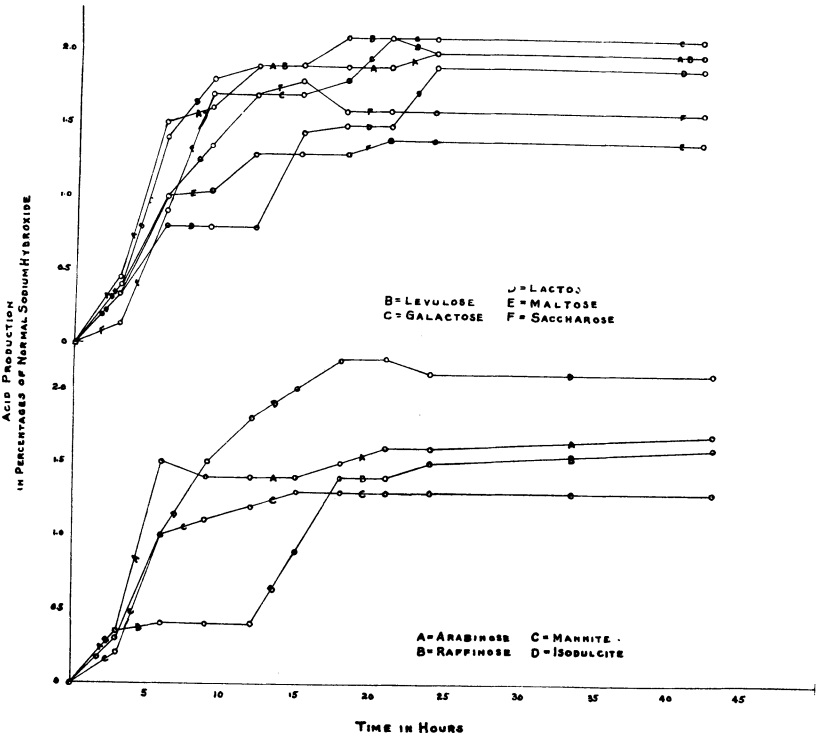

Chart 4.—The relation of time to the amount of acid produced by the bacillus coli, isolated from oysters.

with a neutral or slightly alkaline reaction. Hence, in a medium which is alkaline, the bacillus coli group will produce more acid than in a medium which is slightly acid, but in the end they will both reach the same goal — the maximum acidity of the organism. No acid is produced in a medium with over 2.4 percent acidity. The limit of alkalinity from which the organisms will produce acidity, was not determined.

TABLE 7

TOTAL AMOUNT ACID, IN PERCENT NORMAL, PRODUCED IN VARIOUS CARBOHYDRATES BY THE BACILLUS COLI, ISOLATED FROM FECES

Days	Percentage of Acid with Dextrose	Percentage of Acid with Levulose	Percentage of Acid with Galactose	Percentage of Acid with Lactose	Percentage of Acid with Maltose	Percentage of Acid with Saccharose	Percentage of Acid with Xylose	Percentage of Acid with Arabinose	Percentage of Acid with Raffinose	Percentage of Acid with Mannite	Percentage of Acid with Isodulcite
1	3.0	3.2	2.7	2.3	2.7	1.7	2.0	2.7	2.7	2.6	2.9
	2.9	2.9	2.1	2.5	2.3	2.7	2.4	2.8	1.4	2.7	2.6
2	3.0	3.7	2.5	2.7	2.3	2.2	2.4	2.5	2.9	1.3	2.9
	2.9	3.1	2.2	2.8	2.7	2.5	2.8	2.8	2.2	2.5	1.4
3	3.2	1.1	2.3	3.9	1.3	2.3	4.0	1.0	2.0	1.6	1.1
	2.6	2.1	2.5	2.5	0.6	3.7	2.8	2.0	2.0	1.0	1.2
4	0.3	0.3	0.3	0.4	0	1.6	0	0.25	1.0	0	0
	1.3	0.6	1.1	2.1	0.4	0.4	0	1.6	1.8	0	0.8
5	0.3	0.3	0.2	0.3	0	1.5	0	0.2	0.8	0	0
	0.3	0.3	0.5	0.8	0.5	0.3	0	0.4	0.5	0.2	0.6
6	0.2	0.2	0.3	0.3	0	1.35	0	0	0.4	0.4	0
	0.6	0.3	0.3	0.4	0.2	0.3	0	0	0.4	0.2	0.8
7	0.2	0	0	0.2	0	0.8	0	0	0.3	0	0
	0.4	0.2	0.3	0.4	0.7	0	0	0	0.3	0.2	0.8
8	0	0	0	0.4	0	0.5	0	0	0.2	0	0
	0	0	0.6	0	0	0	0	0	0	0	0.4
9	0	0	0	0	0	0.5	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0.1
10	0	0	0	0	0	0.2	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0
Total Average	10.6	9.15	8.95	11.0	6.85	11.7	8.2	7.62	9.45	6.35	7.3

TABLE 8

COMPARISON BETWEEN TOTAL ACID PRODUCTION AND THE MAXIMUM ACID PRODUCTION OF 24 HOURS INCUBATION

	Dextrose	Levulose	Galactose	Arabinose	Xylose	Mannite	Isodulcite	Lactose	Maltose	Saccharose	Raffinose
Total acid production	10.6	9.15	8.95	7.6	8.2	6.35	7.3	11.0	6.85	11.27	9.45
Maximum acid production of 24 hours	2.35	2.25	1.97	1.87	1.90	2.00	1.90	1.85	2.05	1.35	1.47

ACID PRODUCTION IN A MEDIUM PERIODICALLY NEUTRALIZED TO REMOVE THE ACIDS FORMED

Erlenmeyer flasks containing 100 c.c. of the various carbohydrates (1 percent concentration) were inoculated with 0.5 c.c. of a twenty-four-hour peptone culture of the different members of the bacillus coli group. Before inoculations were made, 0.5 c.c. of phenolphthalein (5 gm. of the commercial salt to one liter of 5 percent alcohol) was added as an indicator. The flasks were incubated at 37 C. and at intervals of 24 hours, the flasks were removed and sterile normal sodium hydroxid was added from a burette, until a faint pink color was obtained. The cultures were replaced in the incubator and the process was repeated at the end of every 24 hours, until no more acid was produced by the organisms.

Table 8 shows that the maximum acid production of 24 hours, which is, as previously proved, the greatest amount which the organism can produce at any one time, is limited by the excess of acid formed, and can be greatly increased by periodical neutralization.

The maximum amount of acid, which the bacillus coli group is able to produce in 1 percent carbohydrate solutions in 24 hours can be greatly increased if the excess of acid formed is neutralized. In all carbohydrates of 1 percent concentration, the total acid production is from 3 to 6 times as large as the maximum twenty-four-hour production which, as previously proved, is the same for 48 and 72 hours under ordinary conditions. From these experiments it may be assumed that acid production goes on until a maximum is reached (24 hours) and then ceases until some of the acid present is neutralized, when acid is again produced until the maximum is reached. This same thing occurs until all the carbohydrates are used. None of the cultures on the twelfth day gave a positive reaction for sugar, showing that the carbohydrates had been used entirely by the organisms in the production of acid. The total amount of acid an organism is able to produce, if the excess acid is neutralized, depends, in a large part, on how much fermentable carbohydrate there is present in the medium. The maximum twenty-four-hour production on the other hand, will use only that amount which is necessary to produce the maximum amount of acid which can be tolerated.

THE AMOUNT OF ACID PRODUCED IN VARIOUS CARBOHYDRATES

An attempt has been made to draw a comparison between the various carbohydrates by the amount of acid produced from them by different members of the bacillus coli group.

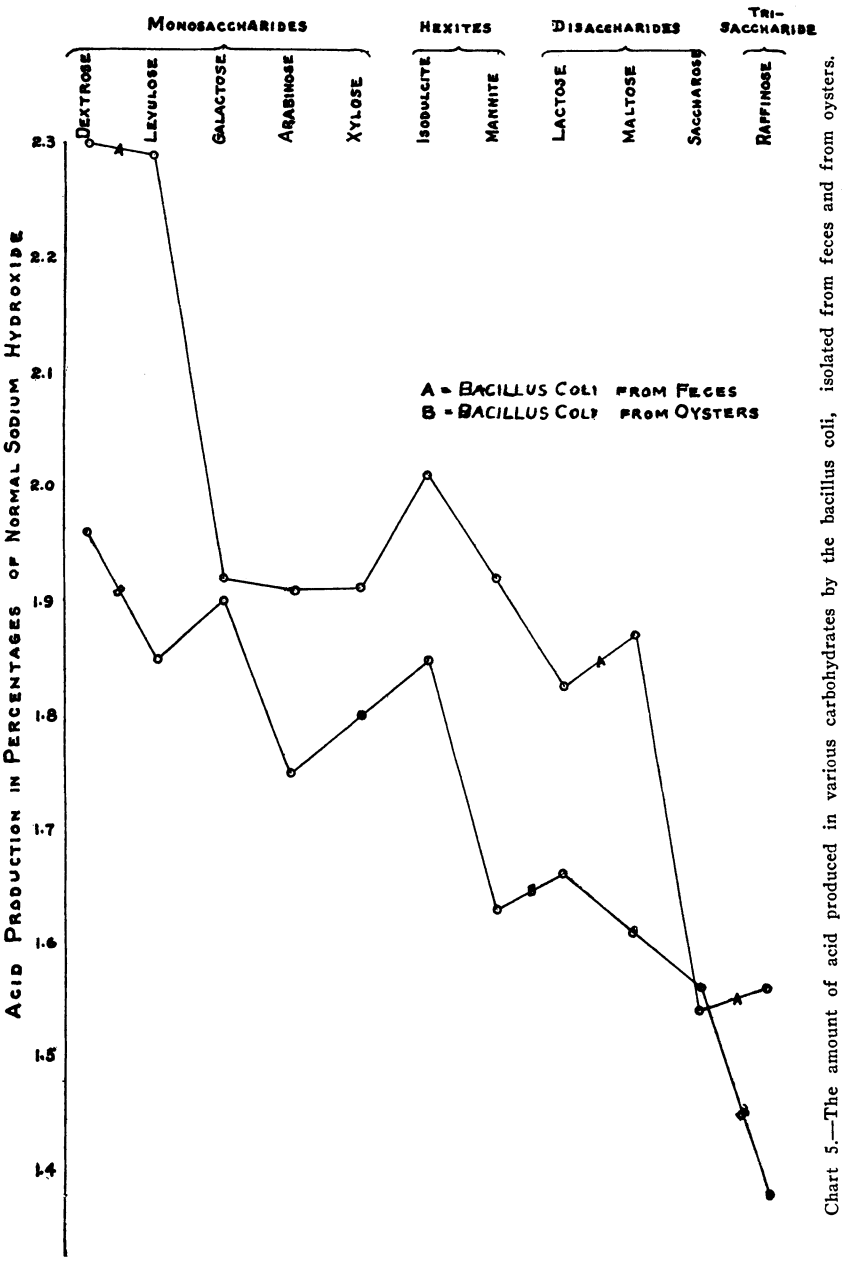

Chart 5.—The amount of acid produced in various carbohydrates by the bacillus coli, isolated from feces and from oysters.

Tubes containing 25 c.c., of the various carbohydrates, were inoculated with 0.5 c.c. of a twenty-four-hour culture of the different members of the bacillus coli group. After 24 hours incubation at 37 C., the cultures were titrated with N/20 sodium hydroxid. In each case strains were selected which could ferment the carbohyrate in question.

Tables 9 and 10 show that the colon bacillus, whether isolated from feces or oysters, produces the maximum amount of acid in the monosaccharids, less in the disaccharids, and the least in the trisaccharid.

The results obtained in this series of experiments show that the various members of the bacillus coli group are able to produce more acid in solutions containing carbohydrates of simple chemical structure than in solutions containing carbohydrates of complex chemical structure. For instance, more acid is produced in solutions containing the monosaccharids and hexites, such as dextrose, levulose, galactose, xylose, arabinose, mannite, and isodulcite than in solutions containing the disaccharids, lactose, maltose, and saccharose. The disaccharids are, in turn, more easily fermented by the bacillus coli group than the trisaccharid raffinose.

The polysaccharid starch is not fermented by any of the members of the coli group. The trisaccharid raffinose, while not so complex a molecule as starch, appears to offer chemically greater difficulties to the organisms in the carrying out of their oxidative processes in the formation of the various acids from the sugars than the organisms would encounter when fermenting, for instance, a disaccharid. In fact, there are members of this group which are unable to ferment raffinose at all.

The arrangement of the carbohydrates, according to the amounts of acid which the members of the bacillus coli group are able to produce from them, agrees somewhat with an arrangement made according to their chemical complexity of structure, as may be seen by the following:

Monosaccharids, 2.03, 1.82.
Disaccharids, 1.74, 1.61.
Trisaccharid, 1.56, 1.38.
Polysaccharid (Starch), 0, 0.

Among the monosaccharids, the hexoses, dextrose, and levulose, seem to be most easily oxidized by the organism with the formation of acid. No distinct difference could be noted in the amounts of acid produced in the levulose and dextrose by the methods used in this

TABLE 9

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED IN VARIOUS CARBOHYDRATES BY BACILLUS COLI, ISOLATED FROM FECES

Carbohydrates	Cultures									
	1	2	3	4	5	6	7	8	9	Average
Dextrose	2.3	2.4	2.4	2.2	2.4	2.3	2.3	2.2	2.2	2.3
Levulose	2.25	2.3	2.4	2.4	2.4	2.1	2.4	2.1	2.3	2.29
Galactose	1.5	1.9	1.95	1.9	1.95	2.0	2.1	1.8	2.2	1.92
Arabinose	1.75	2.2	2.1	1.7	2.05	1.7	...	...	...	1.91
Xylose	1.79	1.9	2.1	2.0	2.0	1.8	...	...	...	1.91
Isodulcite	2.1	2.2	2.2	2.0	2.0	1.4	2.1	2.1	2.0	2.01
Mannite	1.7	2.0	1.9	1.7	2.0	2.0	2.0	2.0	2.0	1.92
Average of all Monosaccharids and Hexites				...	...	...	...	...	...	2.03
Lactose	1.7	1.9	1.9	2.0	1.8	1.9	1.7	1.7	1.89	1.82
Maltose	1.1	1.8	2.15	2.2	2.2	1.9	2.2	1.5	1.8	1.87
Saccharose	1.2	1.85	1.5	1.9	1.1	1.6	1.6	1.6	1.5	1.54
Average of all Dissaccharids			...	...	...	...	...	...	...	1.74
Raffinose	1.4	1.7	1.8	1.6	1.75	1.2	1.6	1.4	1.6	1.56
Average of Trisaccharid			...	...	...	...	...	...	...	1.56

investigation. The amount of acid produced for these sugars also was very constant. Galactose, while not forming so much acid as dextrose and levulose, showed a greater variance in the amount of acid produced. All organisms investigated produced acid in the monosaccharids mentioned.

TABLE 10

ACID, IN PERCENT NORMAL, PRODUCED IN VARIOUS CARBOHYDRATES BY BACILLUS COLI, ISOLATED FROM OYSTERS

Carbohydrates	Cultures						
	1	2	3	4	5	6	Average
Dextrose	2.0	1.9	1.8	2.0	2.2	1.9	1.96
Levulose	1.9	1.6	1.7	1.6	2.2	2.1	1.85
Galactose	2.0	1.9	1.8	2.0	1.9	1.8	1.9
Arabinose	...	...	1.8	1.8	1.9	1.5	1.75
Xylose	1.8	...	1.8	1.8	...	1.8	1.8
Isodulcite	1.7	1.9	1.9	1.6	1.8	2.2	1.85
Mannite	2.0	1.5	1.6	1.6	1.8	1.3	1.63
Average of all Monosaccharids and Hexites			...	...	...	...	1.82
Lactose	1.9	1.6	1.6	1.8	1.8	1.3	1.66
Maltose	1.9	1.5	2.0	1.4	1.6	1.3	1.61
Saccharose	1.7	1.2	1.6	1.5	1.8	1.6	1.56
Average of all Disaccharids			...	...	...		1.61
Raffinose	1.2	1.7	1.4	0	1.2	1.4	1.38
Average of Trisaccharid.			...	...	...		1.38

Tables 9 and 10 show that the colon bacillus, whether isolated from feces or oysters, produces the maximum amount of acid in the monosaccharids, less in the disaccharids, and least in the trisaccharid.

The pentoses, xylose and arabinose, seem to offer greater resistance to the bacterial oxidation processes, as we find less acid produced from the pentoses than from the hexoses. Throughout the work, these two sugars behaved exactly alike, and no differentiation was possible by the amount of acid produced from them by the group.

The hexites, mannite and isodulcite, showed less acid than the hexoses, and at times the amount of acid produced from them exceeded that produced from the pentoses. Many strains of the bacillus coli group were found, which were unable to ferment mannite and isodulcite.

Of the three disaccharids used, saccharose seems to offer greater resistance to the oxidative processes of the colon group than either lactose or maltose. In fact, many members of the group are unable to ferment it. This seems to be in accord with the chemical structure of the sugar, since we know that saccharose is the only one of the three mentioned disaccharids which cannot be oxidized. At times, the amount of acid produced in lactose and maltose equals or even exceeds the amount of acid produced in galactose, a monosaccharid.

The more complicated raffinose offers great difficulties to the fermentative processes of the bacillus coli group. Some strains failed to produce any acid at all from this sugar. As already mentioned, starch was not fermented by any strains used in this investigation.

It should not be understood, of course, that the size of the molecule is the only factor involved, for its configuration is also important. Winslow and Walker (1907) have shown that bacilli of the colon group, which ferment saccharose, usually ferment raffinose as well. The same thing appears in my results as to the amount of acid formed, saccharose results being lower than lactose results tho not quite so low as those obtained in raffinose.

There is an important distinction to be drawn between the power to ferment a given sugar and the amount of acid formed when it is fermented. In my work I have used only strains capable of attacking the sugar in question. When a low result is obtained it may be due to a slow action upon the sugar, or, in view of the evidence that it is the amount of end product formed which usually stops the reaction, it may be that the lower acidity produced in more complex sugars is the result of some other decomposition products, which accompany the acids, and, in connection with them, are able to inhibit growth.

A COMPARISON BETWEEN THE AMOUNT OF ACID PRODUCED BY VARIOUS MEMBERS OF THE BACILLUS COLI GROUP ISOLATED FROM DIFFERENT SOURCES

In this series of experiments, a comparison has been made between the amounts of acid produced in various carbohydrates by the different members of the bacillus coli isolated from three distinct sources: (1) from stools of healthy individuals in and about the laboratory; (2) from stools of Italian immigrants quarantined on board the *S. S. Roma;* (3) from oysters taken from different locations in Narragansett Bay, representing areas of widely diverse characters.

The organisms were inoculated into tubes of peptone broth which were incubated at 37 C. for 24 hours. The various carbohydrates were inoculated by the addition of 0.5 c.c. of this twenty-four-hour culture. At the end of 24 hours' incubation at 37 C. the cultures were titrated with N/20 sodium hydroxid.

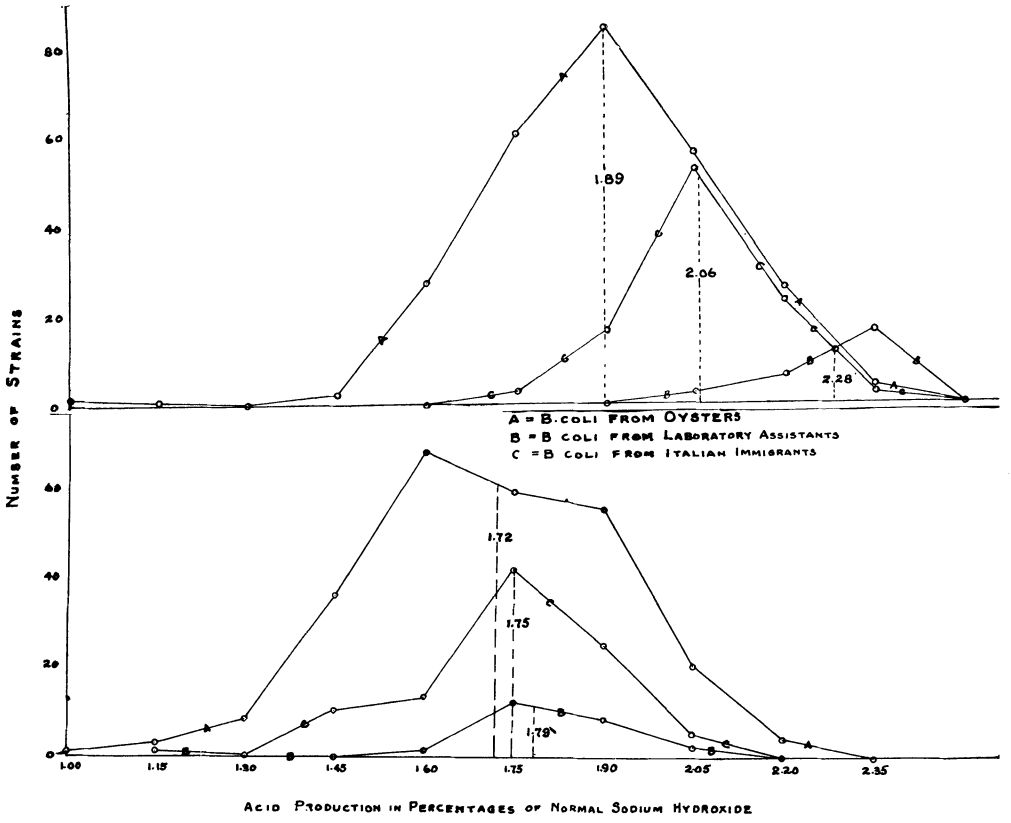

Chart 6.—The amount of acid produced by the bacillus coli in dextrose (upper curves) and lactose broth (lower curves).

The results obtained in this series of experiments seem to show that the source from which the members of the bacillus coli group were isolated has a direct effect upon the ability of the organisms to ferment carbohydrates with the production of acid.

A comparison between the amounts of acid produced in lactose and dextrose broths by members of the bacillus coli group gives the following results:

	From Laboratory Assistants	From Immigrants	From Oysters
Percentage in dextrose	2.28	2.06	1.89
Percentage in lactose	1.79	1.75	1.72

The bacillus coli isolated from feces, both from laboratory assistants and from the immigrants of the *S. S. Roma,* produced more acid in dextrose and lactose broth than the colon bacillus isolated from oysters. This seems to indicate that bacillus coli loses some of its ability to ferment carbohydrate with the production of acid during the journey from the intestinal tract to the oysters. Bacillus coli, isolated from the stools of laboratory assistants, produced more acid in dextrose and lactose broths than similar organisms isolated from the stools of Italian immigrants. The significance of this difference in the ability of the organisms, isolated from the above-named sources, to produce acid from carbohydrates means, is impossible to explain except that the general character of the diet may have had some effect on the ability of the organism to ferment carbohydrates with the production of acid.

EFFECT OF IMMERSION IN SEA WATER ON ABILITY TO PRODUCE ACID

The purpose of this experiment was to determine if the members of the bacillus coli group, after being kept for various periods in sea water, were affected in regard to their ability to produce acid in various carbohydrates.

In these experiments bottles of sea water, which gave two negative tests for the presence of the members of the bacillus coli group with lactose peptone bile, were inoculated with known cultures of the coli group and kept at the following temperatures: ice-box, 4 C.; room, 20 C.

At intervals of a week, portions of the sea water were inoculated into lactose peptone bile tubes, from which the members of the bacillus coli group were isolated and identified. From twenty-four-hour peptone cultures of these organisms, inoculations were made into the various carbohydrate media and incubated 24 hours at 37 C. At the end of that period titrations were made with N/20 sodium hydroxid.

TABLE 11

AMOUNT OF ACID, IN PERCENT NORMAL, PRODUCED IN DEXTROSE AND LACTOSE BROTH BY COLON BACILLI FROM DIFFERENT SOURCES

Source and Number of Cultures	Dextrose			Lactose		
	Lowest	Highest	Average	Lowest	Highest	Average
Italian immigrants, 95	1.75	2.30	2.06	1.40	1.95	1.75
Laboratory assistants, 24	2.10	2.40	2.28	1.20	2.0	1.79
Oysters, 260	1.00	2.40	1.89	1.10	2.25	1.72

TABLE 12

AMOUNT OF ACID PRODUCED IN VARIOUS CARBOHYDRATE MEDIA BY THE BACILLUS COLI RETAINED IN SEA WATER AT 4 C. AND 20 C.

Weeks	Dextrose		Levulose		Galactose		Arabinose		Xylose		Mannite		Lactose		Maltose	
	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.	4 C.	20 C.
1	2.3	2.3	2.3	2.3	1.2	1.2	1.8	1.8	1.9	1.8	2.0	2.0	1.7	1.8	1.6	1.5
2	2.3	2.2	2.25	1.9	1.4	1.2	1.8	1.7	1.9	1.5	2.1	2.0	1.7	1.6	1.5	0.4
3	2.3	2.2	2.3	2.3	1.1	1.2	1.8	1.7	1.7	1.8	2.0	2.0	1.7	1.6	1.7	1.9
4	4.25	2.2	2.3	2.2	1.6	1.6	1.8	1.9	1.95	1.9	2.0	2.1	1.85	1.85	1.7	1.7
5	2.0	2.1	2.3	2.1	1.6	1.4	1.7	1.3	1.9	1.9	1.9	1.9	1.6	1.6	0.6	0.8
6	2.2	2.2	2.3	2.0	1.5	1.7	1.6	2.0	1.9	1.9	2.1	2.0	1.7	1.8	1.8	1.9
7	2.3	1.4	2.3	1.7	1.9	1.6	1.6	2.0	1.9	1.8	1.8	1.7	1.7	1.7	1.5	1.9
8	2.2	...	2.3	...	1.2	...	1.6	...	1.9	...	2.0	...	1.5	...	1.7	...

All results are the averages of two titrations.

These experiments show that the various members of the bacillus coli group, after remaining for 8 weeks in bottles of sea water kept at 4 C. and 20 C., were able to produce the same amount of acid in the various carbohydrates as when first added.

SUMMARY

The optimum temperature for the maximum production of acid in 24 hours by the members of the bacillus coli group, in a medium containing a fermentable carbohydrate, is 37 C. Acid production in this time is almost nil at 3 C., rises rapidly to 37 C., and falls as rapidly above that point, ceasing between 50 C. and 60 C.

Twenty-four hours' incubation at 37 C. is a sufficient period for the maximum production of acid by members of the coli group in a medium containing a fermentable carbohydrate, when 0.5 c.c. of a twenty-four-hour peptone culture is used as an inoculum. Under these conditions no further increase in acidity occurs after 20 hours.

The amount of medium, up to 500 c.c., inoculated with the members of the coli group, has no effect upon the percentage of acidity produced by the organisms, when incubated at 37 C. for 24 hours after inoculation with 0.5 c.c. of a twenty-four-hour peptone culture.

A medium containing 1 percent of fermentable carbohydrate offers a suitable amount of carbohydrate for the maximum acid production by members of the coli group. A medium of much less than 1 percent concentration does not contain enough carbohydrate to bring about the maximum reaction, while a medium of more than 1 percent concentration serves no useful purpose. A high concentration of carbohydrates (over 25 percent) will not only hinder but will prevent the production of acid, but between 1 and 25 percent the twenty-four-hour acid production is the same.

The more distant the initial reaction of the medium is from the maximum acidity necessary to inhibit production of acid, the greater amount of acid the organism can produce before that maximum is reached. The extreme limit of alkalinity, in which the organism will produce acid, was not determined.

The maximum amount of acid, which bacilli of the colon group will produce in ordinary carbohydrate media, is fixed by the tolerance of the bacteria to the acid itself. If the acid formed is neutralized daily by the addition of free alkali, the acid production will go on until all the carbohydrate present has been consumed. With 1 percent carbo-

hydrate in the medium, there will be 4 or 5 times as much acid formed as ordinarily.

The members of the coli group produce the greatest amount of acid in media containing the monosaccharids and hexites, dextrose, levulose, galactose, arabinose, xylose, mannite, and isodulcite; less in the disaccharids, maltose, lactose, and saccharose; and least in the trasaccharid, raffinose.

Members of the bacillus coli group, isolated from feces, produce more acid in media containing fermentable carbohydrates than strains of the bacillus coli group, isolated from oysters taken from different portions of Narragansett Bay. Of the members of the bacillus coli group isolated from feces, strains which were obtained from the stools of laboratory assistants produced more acid in media containing fermentable carbohydrates than strains isolated from the stools of Italian immigrants quarantined aboard the *S. S. Roma.*

The amount of acid produced in various carbohydrate media by the bacilli of the colon group is unaffected by storage in unsterilized sea water for a period of 8 weeks at temperatures of 20 C. and 40 C.